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CASE REPORT

Tenofovir Disoproxil Fumarate Fails to Prevent HIV Acquisition or the Establishment of a Viral Reservoir: Two Case Reports

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ABSTRACT

The use of antiretrovirals as pre-exposure prophylaxis (PrEP) is highly efficacious in HIV prevention. The World Health Organization recently recommended Truvada® (Gilead Sciences, Inc.) or tenofovir disoproxil fumarate (TDF) for high-risk individuals, with limited

data for single-agent TDF PrEP in men who have sex with men (MSM). We report two cases of TDF PrEP failure in MSM who had received long-term TDF for hepatitis B infection and had therapeutic levels of drug immediately after HIV acquisition. Rapid antiretroviral intensification at diagnosis of acute HIV infection failed to limit immune dysfunction or prevent the establishment of a viral reservoir.

On behalf of CHERUB Collaboration.

J. Frater and S. Fidler are joint senior authors and contributed equally.

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INTRODUCTION

The use of antiretrovirals (ARVs) as pre-exposure prophylaxis (PrEP) is highly efficacious at limiting HIV transmission [1–6]. Recent World Health Organization (WHO) guidelines recommend single-agent tenofovir disoproxil fumarate (TDF) or Truvada® [TDF/emtricitabine (FTC); Gilead Sciences, Inc.] for individuals at risk of HIV acquisition [7]. Single-agent TDF PrEP shows benefit over placebo and is comparable with Truvada in preventing HIV transmission in HIV serodiscordant heterosexual couples [2] and individuals who inject drugs [3]. Efficacy data for TDF in men who have sex with men (MSM) are limited [8] and it is not known whether drug level requirements are the same for both HIV treatment and prevention. However, animal model data suggest that TDF alone is less protective than Truvada [9, 10] and pharmacokinetic analysis of the partners PrEP study supports this [11].

Cases of PrEP failure mainly occur due to poor adherence [1–6, 11]. Early detection is essential to minimize monotherapy drug exposure and prevent drug resistance development [12]. Whether ARV therapy (ART) should be stopped or intensified at PrEP failure is unclear [13]. A recent case presentation reported an undetectable viral reservoir in an individual's intensifying ART at PrEP failure [14]. This is supported by cohort data and data from macaques suggest that three-drug ARV at acute HIV infection can improve clinical outcome [15–17] by limiting viral reservoir [17–20] and immune dysfunction [21–23], and that these benefits are greater the earlier the ARVs are started [15–17].

We report two cases of PrEP failure amongst MSM receiving long-term TDF for hepatitis B treatment with therapeutic tenofovir levels around the time of HIV-1 acquisition. Despite

continuing TDF and intensifying to combination ARV at acute HIV diagnosis, both cases had significant viral reservoirs and elevated markers of immune activation/exhaustion within 4 weeks of HIV-1 diagnosis consistent with established HIV-1 infection.

CASE REPORTS

Patients

Informed consent was obtained from both patients for being included in this case report.

Two patients from separate HIV centers were diagnosed with acute HIV whilst receiving TDF monotherapy for hepatitis B infection. To allow case comparison, day 1 was defined as the day of estimated date of HIV seroconversion (EDSC, i.e., the mid-point date between HIV-negative and HIV-positive test or 14 days prior to p24 antigen-positive/HIV antibody-negative result).

Both patients had received TDF 300 mg/day for hepatitis B infection (hepatitis B s-antigen and e-antigen positive) and maintained a consistently undetectable hepatitis B DNA for 3+ years with no viral blips. In the 6 weeks prior

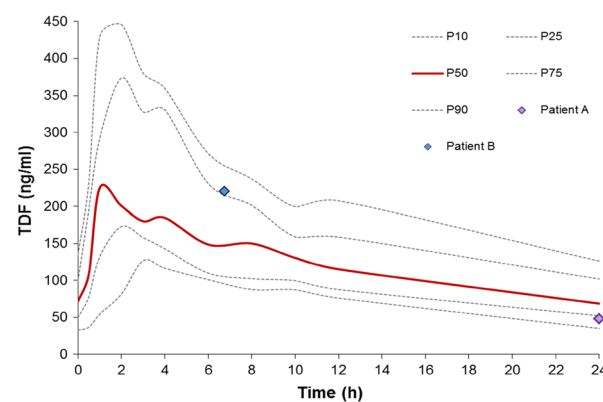


Fig. 1 Pharmacokinetic plot of tenofovir levels taken at HIV diagnosis. For patient A the tenofovir trough level 17 h post-dose was 48 ng/mL (between 10th and 25th centile) and for patient B the tenofovir trough concentration 7 h post-dose was 220 ng/mL (75th centile). TDF tenofovir disoproxil fumarate

to HIV acquisition both individuals reported condom-less receptive anal sex with casual male partners and denied any missed doses of TDF. Longstanding good adherence was further suggested by the regularity of pharmacy script provision and drug levels carried out at HIV diagnosis were within the therapeutic range (Fig. 1). No other sexually transmitted infections were detected at HIV diagnosis. ARV was intensified within 3 weeks of presumed HIV acquisition and blood taken for quantification of viral reservoir and immune function approximately 1 week later. The cases are summarized in Table 1.

Laboratory Results

Patient A was diagnosed with acute HIV infection (HIV antibody positive 10 days after HIV antibody-negative test and by 3 bands on Western Blot) following a 4-day history of mild flu-like symptoms suggestive of seroconversion illness. Results 9 days after EDSC were: CD4 T cell count 584 cells/ μ L (35%), CD4:CD8 ratio 1.19, and HIV-1 plasma viral load <50 copies/mL. The ARV regimen was immediately intensified (12 days from EDSC) to Eviplera[®] (25 mg rilpivirine, 200 mg emtricitabine, and 245 mg TDF; Gilead Sciences International Ltd.) and the viral load remained undetectable thereafter. Viral genotype failed to amplify due to low viral load. Results 15 days after EDSC (3 days after ARV intensification) showed total HIV-1 DNA 1381 copies/million CD4 cells, integrated DNA 586.6 copies/million CD4 T cells, and unspliced intracellular HIV-1 RNA transcripts 116 copies/ 10^6 copies 18 s RNA.

Patient B was diagnosed with acute HIV (p24 antigen-positive/antibody-negative antibody) following hospitalization with a severe seroconversion illness comprising severe flu-like symptoms, fatigue, and myalgia.

Results 14 days after EDSC were: CD4 T cell count 550 cells/ μ L (24%), CD4:CD8 ratio 0.49, and HIV-1 plasma viral load 103,306 copies/mL. The regimen was intensified 19 days after EDSC to Truvada (one tablet once daily), raltegravir (400 mg twice daily), darunavir (800 mg once daily), and ritonavir (100 mg once daily). Viral genotype showed wild-type drug-sensitive virus. Blood taken 24 days after EDSC (5 days after ART intensification) showed total HIV-1 DNA 2746 copies/million CD4 cells, integrated DNA 1431.6 copies/million CD4 T cells, and unspliced intracellular HIV-1 RNA transcripts 1236 copies/ 10^6 copies 18 s RNA.

Immune activation, defined as the percentage expression of CD38 and HLA-DR on CD4 and CD8 T cells, was 65.8 and 57% for patient A and 42.2 and 38.8% for patient B.

METHODS

HIV Diagnosis, Viral Load, and Therapeutic Drug Level Monitoring

HIV testing was carried out using the Abbott Architect HIV Ag/Ab combo assay and VIDAS quantitative HIV p24 11 assay. Confirmation occurred with Vidas HIV Duo Quick (HIV6) ELFA fourth-generation assay and Bispot Immunocomb third-generation assay.

Western blot was carried out using MP Diagnostics HIV Blot 2.2. HIV-1 plasma viral load was quantified by Roche COBAS V2.0, and viral genotype determined using Taqman sequencing. Tenofovir (TFV) drug levels were measured by a validated HPLC–MS/MS with a lower limit of quantification of 5 ng/mL [24]. The tenofovir concentration for each patient was plotted against a percentile plot derived from a published population pharmacokinetic model of tenofovir plasma concentrations in HIV-infected subjects (Fig. 1) [25].

Table 1 Summary characteristics of cases of HIV-1 acquisition on TDF

Characteristics	Patient A	Patient B
Acute HIV diagnosis	HIV-positive test 12 days after HIV-negative test	P24 antigen positive/HIV antibody negative
Hepatitis B history		
Duration known hepatitis B infection	6 years	7 years
Duration TDF monotherapy	4 years	3 years
No. hepatitis B VL blips on TDF	0	0
HIV seroconversion symptoms	Mild fever	Hospitalized with severe sore throat, fever
Blood results at acute HIV diagnosis		
Days from EDSC to blood test	10	14
HIV VL (copies/mL)	<50	158,899
CD4 T cell count	584 (35%)	550 (24%)
CD4:CD8 ratio	1.19	0.49
Hepatitis B VL	Undetectable	Undetectable
HIV genotype	Not possible	Wild type
Intensified ART regime		
Days from EDSC to intensification	15	19
Regime	Eviplera	Truvada, raltegravir, darunavir, ritonavir
Reservoir quantification		
Days from EDSC to blood test	12	24
HIV total DNA (copies/million CD4 cells)	1381 (3.14)	2746 (3.44)
Integrated DNA (copies/million CD4 T cells)	586.6 (2.77)	1431.6 (3.16)
RNA (copies/million CD4 T cells)	116	1236
Immunology		
Days from EDSC to blood test	15	24
CD4 + CD38 + (%)	65.8	42.2
CD4 + HLA-DR + (%)	3.12	4.34
CD8 + CD38 + (%)	57	38.8
CD8 + HLA-DR + (%)	14.4	9.58

ART antiretroviral therapy, *EDSC* estimated date of HIV seroconversion, *TDF* tenofovir disoproxil fumarate, *VL* viral load

HIV Reservoir

Purified CD4 T cells were analyzed by qPCR for HIV-1 DNA (total and integrated) and cell-associated HIV-1 RNA unspliced transcripts (CA-RNA) as reported elsewhere [19].

Immune Activation and Exhaustion

PBMC were stained with the anchor markers (CD3-VioBlue, CD4(VIT4)-VioGreen, CD8-APC) and a Live/Dead marker Near IR-APC-Cy7 plus either an activation panel (CD25(3G10)-PE, CD38-PE-Vio770, CD69-FITC, Anti-HLA-DR-PerCP) or an exhaustion panel (TIGIT-PE, TIM-3-FITC, LAG-3-PerCPeF710, PD1-PE-Cy7). Cells were run on a MACSQuant and analyzed with FlowJo software v10 (Miltenyi Biotec).

DISCUSSION

As PrEP is becoming more widely available and uptake increasing, this is a timely reminder that TDF monotherapy PrEP in MSM has limited efficacy data and that HIV-1 acquisition can occur in the presence of TFV drug levels within the therapeutic range required to treat HIV [26]. These cases are instructive to providers and the field of PrEP, and highlight that patients with HBV receiving tenofovir for HBV should consider intensification to Truvada (TDF/FTC) if they meet guidelines (e.g., Centers for Disease Control and Prevention) for PrEP. The lack of resistance detected concurs with randomized control study data showing a very low incidence of resistance in cases of tenofovir failure [29].

Although PrEP effectiveness is largely driven by adherence [1–8, 11, 26, 27], this was not implicated in these cases as evidenced by consistently undetectable hepatitis B DNA and therapeutic plasma levels of tenofovir at HIV

diagnosis (a proxy for HIV acquisition). Whilst drug levels were high enough to treat both hepatitis B and HIV infection [26], the drug level required to protect from HIV infection is not known and, if higher, may explain the transmission events. Additionally, it is not known whether hepatitis B increases susceptibility to HIV and the presence of TDF-resistant mutations may need to be excluded by minor variant sequencing [12].

These cases also show that rapid intensification with ARVs did not prevent reservoir seeding or immune dysfunction. Indeed, the level of viral reservoir, immune activation, and immune exhaustion were comparable to those observed in untreated primary HIV cohorts [15]. Patient A is of additional interest as this occurred despite no evidence of on-going viral replication in plasma prior to ARV intensification. This suggests the importance of sanctuary sites for the establishment of viral reservoir and is consistent with primate models [27].

CONCLUSION

Single-drug TDF PrEP was not effective in preventing HIV infection and intensified ART post-infection did not reduce the viral reservoir in either patient. Whilst single-agent PrEP is not to be recommended as an HIV prevention strategy in MSM in European [28] and US [13] PrEP guidelines, it is by the WHO [7]. Close monitoring of outcomes of TDF usage in MSM needs to be carried out.

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Compliance with Ethics Guidelines. Informed consent was obtained from both patients for being included in this case report.

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